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Providing a reaction tube that contains a first reaction mixture having a first primer and deoxynucleoside triphosphates in a top portion of the tube, a second reaction mixture having a second primer and a DNA polymerase in a lower portion of the tube, and a layer of wax separating the first reaction mix from the second reaction mixture

<u>102</u>

Adding a biological sample to the first reaction mixture and incubating the first reaction mixture under conditions suitable for a telomerase to produce an extension product from the first primer

104

Elongating the extension product

<u>106</u>

Admixing the extension product with the second reaction mixture by melting the wax layer

108

Amplifying the extension product by real-time PCR

110

Quantifying the amplified extension product using a control template

<u>112</u>

100

Fig. 1

XXTTAGGGXXXXXXXX (1st primer)

1

Telomerase-mediated

XXTTAGGGXXXXXXXXXXX(TTAGGG),TTAGGGTTAGGG

(extension product)

XXTTAGGGXXXXXXXX (unextended 1st primer)

1

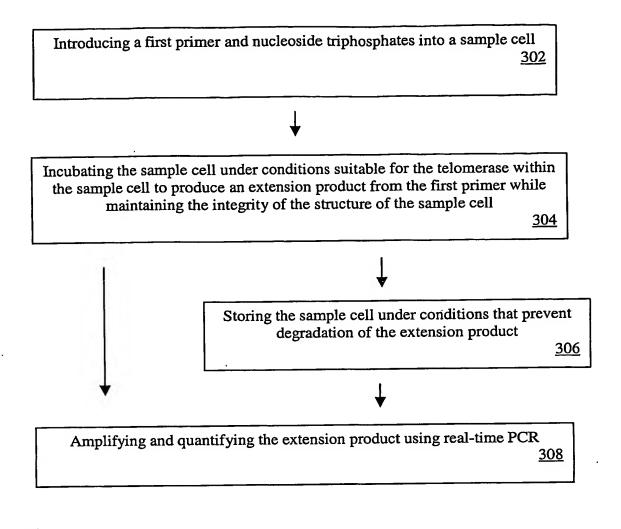
Template-independent elongation

XXTTAGGGXXXXXXXXX(TTAGGG),TTAGGGTTAGGGAAAAAAAAAA(A)_m (elongated ext. product)
AATCCCAATCCCTTTTTTTTT (2nd primer)

. XXTTAGGGXXXXXXXAAAAAAAAAAAA(A)_m (elongated 1st primer)
TTTTTTTTT (2nd primer)

AATCCCAATCC

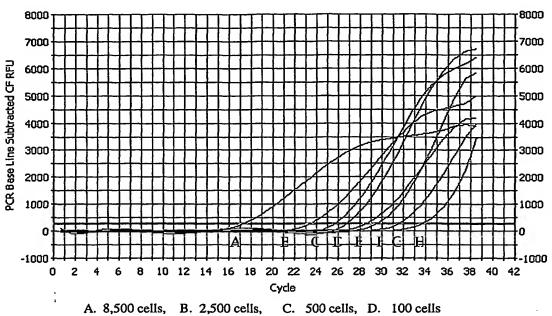
Fig. 2



300

Fig. 3

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- C. 500 cells, D. 100 cells

- E. 20 cells,
- F. 4 cells,
- G. 1 cell, H. Blank.

Fig. 4A

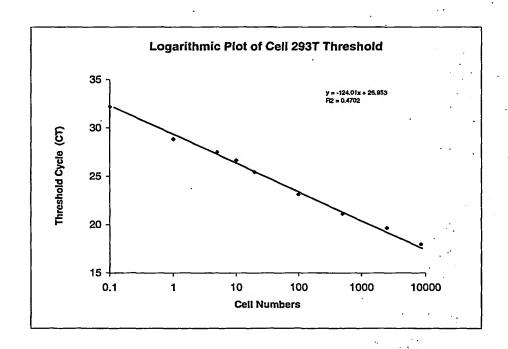
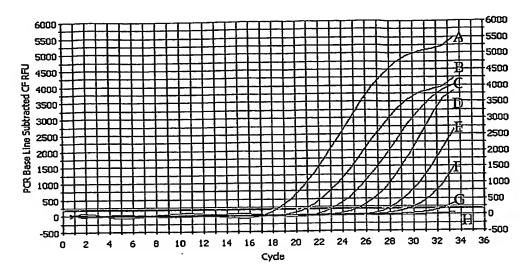


Fig. 4B

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- A. 300,000 molecules, B. 60,000 molecules,
- C. 12,000 molecules,

- D. 2,400 molecules,
- E. 480 molecules,
- F. 100 molecules,

- G. 20 molecules,
- H. Blank.

Fig. 5A

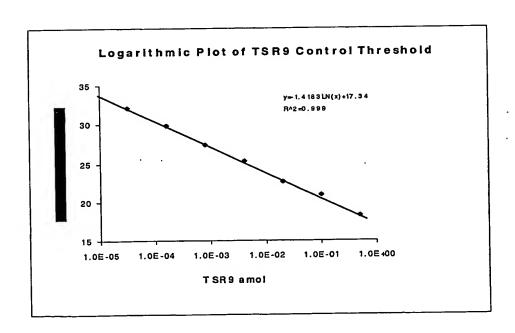
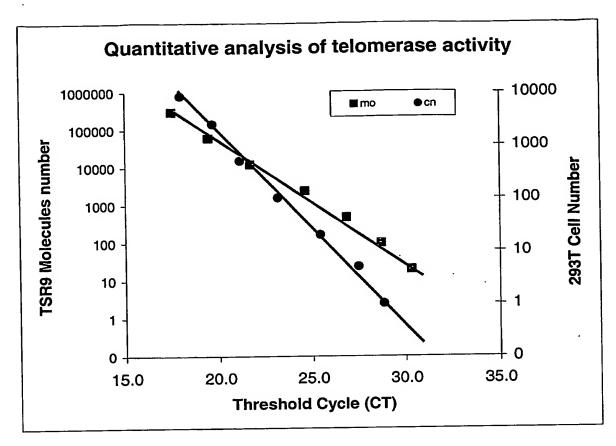


Fig. 5B



mo: Linear regression between TSR9 molecule number and threshold cycle, cn: Linear regression between 293T cell number and threshold cycle.

Fig. 6

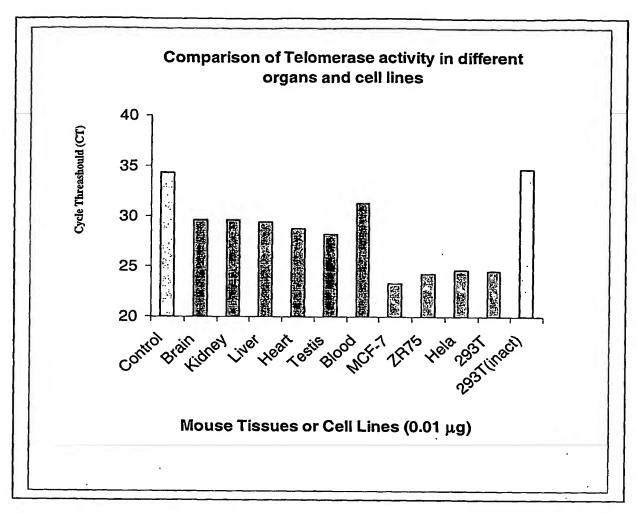
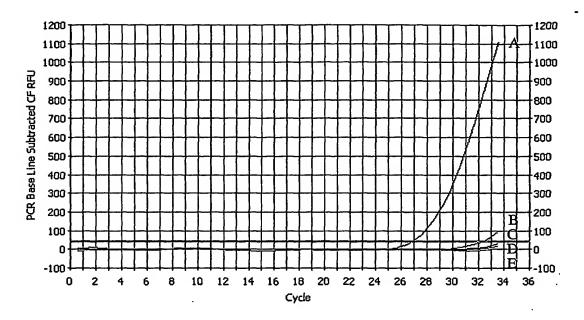


Fig. 7

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A: syringe-treated cells incubated with TS prime;

C: untreated cells incubated with TS primer

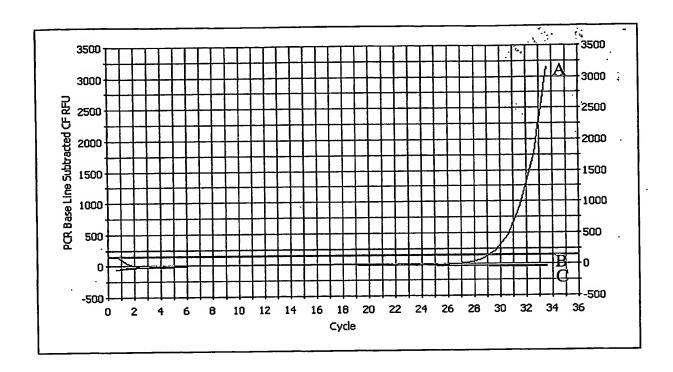
E: blank.

B: syringe-treated cells incubated without TS primer;

D: untreated cells incubated without TS primer;

Fig. 8





A; with TS primer, B: without TS primer, C: blank

Fig. 9